PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/71

A1

(11) International Publication Number: WO 94/05300

(43) International Publication Date: 17 March 1994 (17.03.94)

(21) International Application Number: PCT/CA93/00384

(22) International Filing Date: 2 September 1993 (02.09.93)

(30) Priority data:

938,774	3 September 1992 (03.09.92)	US
102,822	6 August 1993 (06.08.93)	US

(60) Parent Applications or Grants
(63) Related by Continuation
US 938,774 (CIP)
Filed on 3 September 1992 (03.09.92)
US 102,822 (CIP)
Filed on 6 August 1993 (06.08.93)

(71) Applicant *(for all designated States except US)*: BIOCHEM PHARMA INC. [CA/CA]; 2550 Daniel Johnson Boulevard, Suite 600, Laval, Quebec H7T 2L1 (CA).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : VEZINA, Claude [CA/CA]; 11 St.-Sulpice Street, Oka, Quebec J0N 1E0 (CA).

(74) Agent: BERESKIN & PARR; 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: USE OF RAPAMYCIN IN THE TREATMENT OF AIDS

(57) Abstract

The present invention relates to the use of rapamycin or an analog of rapamycin in the manufacture of a medicament for treating, arresting the development or retarding the progression of an HIV infection in an amount sufficient to achieve a reduction in the level of serum p24 antigen; and to the use of rapamycin or analog of rapamycin for treating, arresting the development or retarding the progression of AIDS in a mammal. The invention also relates to a method for treating, arresting the development or retarding the progression of AIDS in a mammal in need thereof by administering to the mammal an amount of rapamycin or analog of rapamycin sufficient to achieve a reduction in the level of serum p24 antigen.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

USE OF RAPAMYCIN IN THE TREATMENT OF AIDS

BACKGROUND OF THE INVENTION

1. Field of the invention

This invention relates to the use of Rapamycin and analogs as anti-viral agents through suppression of immune host cells.

2. Description of the Prior Art

Rapamycin is an anti-fungal antibiotic described by C. Vezina et al., J. Antibiot., 28, 721 (1975) and S.N. Sehgal et al., U.S. Pat. No. 3,929,992, issued Dec. 30, 1975. Rapamycin is extracted from Streptomyces hygroscopicus isolated from an Easter Island soil sample and is particularly effective against Candida albicans both *in vitro* and *in vivo*.

In addition, a report by R.R. Martel et al., Can. J. Physiol., 55, 48 (1977) describes the use of rapamycin for the prevention of the development of two experimental immunopathies [experimental allergic encephalomyetis (EAE) and adjuvant arthritis (AA)]. This report also describes the inhibitory effect of rapamycin on the formation of humoral (IgE-like) antibody (immunosuppressive activity).

Rapamycin has further been reported to be an immunosuppressant by Wartner et al.. They reported that rapamycin may be used to treat or retard the evolution of systemic lupus erythematosus (SLE) (U.S. Pat. No.5,078,999). Sturm et al. have reported that rapamycin may be used to treat pulmonary inflammation (U.S. 5,080,899). WO 92/08474 also describes the use of rapamycin as an immunosuppressant in the treatment of lung disease.

Rapamycin has also been reported to be an anti-tumor agent is certain malignant diseases (U.S. 4,401,653; 4,885,171; 5,066,493).

Several analogs of rapamycin have since been reported to possess some immunosuppressive activity: mono- or diacyl rapamycin (U.S. 4,316,885); hydrogenated derivatives of rapamycin (U.S. 5,023,262); 42-oxorapamycin (U.S. 5,023,263); 27-oxime rapamycin (U.S. 5,023,267); rapamycin pro-drugs (U.S. 4,650,803); 42-oxime rapamycin (U.S. 5,100,883); silyl esters of rapamycin (U.S. 5,120,842); rapamycin dimers (U.S. 5,120,727); rapamycin hydrazone (U.S. 5,120,726); bicyclic rapamycin (U.S. 5,120,725); carbamates of rapamycin (U.S. 5,118,678); amide esters of rapamycin (U.S. 5,118,677); 15-hydroxy- and 15,27-hydroxyrapamycin (U.S. 5,102,876); carboxylic acid esters of rapamycin (WO 92/05179); fluorinated esters of rapamycin (U.S. 5,100,883); 29-desmethyl rapamycin (U.S. 5,091,389); and 7, 29-bisdesmethyl rapamycin (U.S. 5,093,338), all of which patents are included herein by reference.

Ondeyka et al. (U.S. 5,091,389) and Byrne et al. (U.S. 5,093,338) suggest that certain rapamycin analogs (29-desmethyl and 7, 29-bisdesmethyl, respectively) may be useful for the treatment of the <u>auto-immune component of AIDS</u>. However, its action on the virus replication itself has not been suggested.

PCT patent publication WO 93/14780 discloses the use of immunosuppressors, such a cyclosporin A and FK 506 for the manufacture of a medicament against AIDS. Nowak also describes avenues of immunosuppressive therapy in the treatment of AIDS (The Journal of NIH Research, June 1993, vol.5, p.54-58). Such an approach focuses on the treatment of the auto-immune component of AIDS by preventing

activation of T cells, thereby preventing damages done to the immune system by the virus.

The present inventor now addresses the HIV replication problem by eliminating the cellular reservoir of host cells ($CD4^+$ monocytes, macrophages and lymphocytes) of HIV by using cytotoxic doses of rapamycin for limited periods of time. Nowhere has there ever been an indication or suggestion that rapamycin may have an effect on the virus presence itself through selective suppression of the host cell population.

In fact, the present inventor had experimented on the *in vitro* anti-HIV efficacy of rapamycin on the reverse transcriptase activity of HIV-infected T lymphocytes with negative result, leading him to believe that rapamycin did not possess any anti-HIV activity.

The inventor has now found that rapamycin does have anti-HIV activity when assessed *in vivo* in HIV-infected tumor bearing nude mice. Such activity is revealed by dramatic decreases in the level of p24 serum antigen.

Subsequent *in vitro* experiments revealed that this anti-HIV effect was caused by the selective suppression of HIV-host $CD4^+$ cells, thereby preventing the virus from replicating.

Such toxicity may also be used against any other type of infectious agent that would infect immune cells.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided the use of Rapamycin or analogs thereof in the manufacture of a medicament for treating, arresting the development or retarding the progression of HIV infection in an amount sufficient to achieve a reduction in the level of serum p24 antigen.

According to a further aspect of this invention, there is provided a method for treating, arresting the development or retarding the progression of acquired immunodeficiency syndrome (AIDS) in a mammal which comprises administering to the mammal an effective amount of rapamycin or analog thereof to achieve a decrease in p24 antigen level in the serum of said mammal.

According to another aspect of the invention, there is provided the use of rapamycin for treating, arresting the development or retarding the progression of AIDS in a mammal.

According to a further aspect of the inveniton, there is provided a method for treating, arresting the development or retarding the progression of AIDS in a mammal in need thereof which comprises administering to the mammal an anti-HIV effective amount of rapamycin or an analog thereof.

DESCRIPTION OF THE FIGURES

Figure 1 is a bar graph representing the amount of p24 antigen found in the serum of animals treated in accordance with the invention;

Figure 2 is a bar graph representing the tumor size found in animals treated in accordance with the invention;

Figure 3 is a graph representing the effects of rapamycin on the viral inhibition and cellular proliferation on CEM cells acutely infected with HIV-1;

Figure 4 is a graph representing the effects of AZT on the viral inhibition and cellular proliferation on CEM cells acutely infected with HIV-1;

Figure 5 is a graph representing the effects of rapamycin on the viral inhibition and cellular proliferation on U937 cells acutely infected with HIV-1; and

Figure 6 is a graph representing the effects of AZT on the viral inhibition and cellular proliferation on U937 cells acutely infected with HIV-1.

DETAILED DESCRIPTION OF THE INVENTION

According to the present method, rapamycin or one of its analogs is employed as the active ingredient. The isolation and description of rapamycin is given in U.S. Pat. No. 3,929,992, cited above, herein incorporated by reference. The production of rapamycin analogs is given in U.S. 4,316,885; U.S. 5,023,262; U.S. 5,023,263; U.S. 5,023,267; U.S. 4,650,803; U.S. 5,100,883; U.S. 5,120,842; U.S. 5,120,727; U.S. 5,120,726; U.S. 5,120,725; U.S. 5,118,678; U.S. 5,118,677; U.S. 5,102,876; WO 92/05179; U.S. 5,100,883; U.S. 5,091,389; and U.S. 5,093,338, all of which patents are included herein by reference.

In the present invention, Rapamycin was either:
1) administered to virally-infected tumorigenic T-lymphocytes injected into athymic nude mice for the purpose of evaluating the serum p24 antigen levels, or

2) incubated *in vitro* with $CD4^+$ T-lymphocytes and monocytes uninfected or infected with a defective virus (HIV-1 HTLV III_B R3 strain) to evaluate its anti-viral and cytotoxic effects.

3) incubated in vitro with CD4 T-lymphocytes and monocytes acutely infected with HIV-1 HTLV III_B strain to evaluate its anti-viral and cytotoxic effects.

While rapamycin or its analog can be administered as the sole component, it is preferred to formulate the compound in various unit dosage forms for oral or parenteral administration, e.g. tablets or sterile solutions. Such formulations are described in U.S. Pat. No. 3,929,992 cited above. Also, see U.S. Patents 5.066,493, and 4,401,653 incorporated herein by reference. Preferably, the total dose of rapamycin should be higher than the ones used for long-term immunosuppressive therapy in humans (ranging from 0.25 mg/kg/day to 5 mg/kg/day). Preferred higher doses range from 5 to 500 mg per kg of body weight per day with a most preferred dosage range from 10 to 250 mg per kg per day.

Rapamycin or its analogs may also be administered in combination with a therapeutically effective amount of an anti-viral agent useful in the treatment of AIDS. Such agents include reverse transcriptase inhibitors, protease inhibitors, or immunomodulators. Preferably, this anti-HIV agent is a reverse transcriptase inhibitor. Most preferably the reverse transcriptase inhibitor is selected from AZT, d4T, ddI, ddC, 3TC or analogs of 3TC and is present in an amount effective to inhibit HIV replication.

EXAMPLES

EXAMPLE 1.

Administration of rapamycin to virally-infected tumorigenic T-lymphocytes injected to athymic nude mice.

EXPERIMENTAL METHOD:

Cell line and HIV culture.

CCRF-CEM or CEM, a well characterized tumorigenic and HIV permissive cell line, is acquired from the American Type Culture Collection, Rockville, Md. (ATCC CCL119) and maintained in RPMI 1640 medium with 15% heat inactivated fetal calf serum and 50 μg/mL gentamicin. The cells are propagated at 37°C in a humid 5% CO_2 atmosphere. Stocks of the HIV-1 isolates HTLV-III_B are acquired from the NIH AIDS Research and Reference Reagent Program (catalog no. 398), and are harvested from cultures of chronically-infected CCRF-CEM cells. For routine propagation, virus containing culture fluids are clarified of cells by low speed centrifugation and passed through 0.45 μm filters. Infectious virions are quantitated on MT-2 cells in microculture using cytophatic effect (CPE) as end point for infection. The 50% tissue culture infectious dose ($TCID_{50}$) is calculated by the method of Reed and Muench (Amer. J. Hygiene 1938; 27, 493-497). CCRF-CEM cells used for virus xenotransplantation are acutely infected with stock dilution of HIV-1 at MOI (input multiplicity of infection) of 0.1 followed by adsorption for 2 hours at 37°C.

Animals and Environment.

Outbred athymic, 27 ± 2 days old female nude (nu/nu) mice were purchased from Harlyn Sprague Dawley, Inc. The athymic mice are housed at 23 ± 1°C, without antibiotic coverage, in a specific pathogen-free room under laminar flow HEPA-filtered air. All bedding, cages, water, and other material coming in direct contact with the mice were

autoclaved before use. Animals were permitted access to food and water *ad libitum*.

Before treatment, the animals are exposed to 500 Rad's of ^{137}Cs irradiation to reduce natural killer cell activity. The next day the drug injection is started as follows:

Preparation and Administration of Compounds.
Doses of rapamycin are freshly prepared for each day's drug administration. 100 mg/kg of body weight are first weighted out then dissolved in physiological saline containing 0.01% Tween-80®. The drug is then transferred to a small glass Dounce tissue homogenizer of 7 mL volume and then micronized using 10 strokes of the "B" tolerance plunger. After visually determining that a solution is achieved, 0.2 mL of the appropriate mixture is administered to each mouse by gavage (i.g.).

AZT is procured as over-the-counter RETROVIR® 100 mg capsules. The contents from each capsule is dissolved in 200 mL of sterile distilled water by mixing on a stir plate for one hour. The inactive filler component of the capsule contents is removed by centrifugation at 3,000 rpm for 10 minutes. The supernatant is adjusted with additional sterile distilled water to achieve a final concentration of 0.125 mg/mL. The animals are monitored daily to assure an average comsumption of 4mL/day/mouse or 20 mg/kg/day. AZT is prepared fresh on a weekly basis to maintain stability.

On the following day the animals are inoculated with cells as follows:

Cell/HIV Transplantation.
CEM cell cultures, both HIV infected and uninfected, are harvested and washed with serum free media and reharvested. Inocula are standardized by counting the

cells with a hemocytometer, and adjusting the cell suspension with serum free medium. Cells suspended in 0.2 mL media are injected subcutaneously (s.c.) into the intrascapular region of the mouse using a catheter. This catheter is first tunneled through the subcutaneous tissues to eliminate leakage of the inoculum. Aseptic procedures are used throughout.

The mice are observed the following day and at least three times a week for the duration of the experiment. At each of these time points, the inoculation site is examined, and the tumor size is measured in two dimensions via calipers. The tumor volume is calculated from measurements in two dimensions using the formula for a prolate ellipsoid, $\pi/6\ LW^2$. At the termination of the experiment, the animals are placed under methoxyflurane anesthesia. After the pain response is absent, the animal is bled by cardiac puncture until dead. Upon termination of the experiment and animal sacrifice, serum p24 antigen levels are determined as an indication of HIV infection in transplanted CEM cells.

p24 Enzyme Immunoassay.

The p24 enzyme immunoassay (EIA) used is the unmodified procedure commercially available from Coulter Corporation (Hialeah, Fl), which uses a murine monoclonal antibody to the HIV core protein coated into microwell strips. The assay detects p24 gag antigen in culture supernatants, plasma, and serum. Non-specific cross reactions with mouse serum are not seen with this assay.

EXPERIMENTAL DESIGN

The *in vivo* assay using this model consisted of testing the rapamycin in a prophylactic manner, whereby a single dose of agent is administered to one group of mice one day prior to HIV challenge and repeated every other day. The

test and control animals are followed for a minimum period of 28 days. The specific experimental design consists in the comparison of the following groups:

Cell control (Group No.1) - 12 mice transplanted with human CCRF-CEM cells only

Virus control (Group No.2) - 12 mice transplanted with CCRF-CEM infected with HIV at a MOI of 0.1, placebo treated (saline + Tween 80)

Drug control (Group No.3) - 7 mice treated with rapamycin, at 100 mg/kg/day administered by gavage (i.g.) every other day for an average dose of 50 mg/kg/day, transplanted with CCRF-CEM cells (without virus)

AZT control (Group No.4) - 7 mice treated with AZT (20 mg/kg/day), challenged with HIV at the same MOI as the virus control

Rapamycin (Group No.5) - 7 mice treated with rapamycin at 100 mg/kg/every other day administered by gavage (i.g.) for an average dose of 50 mg/kg/day, challenged with HIV at the same MOI as the virus control

Serum p24 antigen levels were determined as the experimental endpoint. Table 1 presents the result of the serum p24 assay in each group.

Table 1 and Figure 1 show the anticipated p24 suppression found in AZT treated group (No.4) when compared to the untreated virus control group (No.2). Moreover, the results of the rapamycin treated group (No.5) shows an apparent reduction in p24 serum levels. It can be anticipated that modulation in oral doses (lower or higher) would yield significant reduction in p24 levels when compared to the untreated virus controls.

Table 1. Mean Group Serum p24 (ng/mL)

Group No.	1	2	3	4	5	6	7	8	9	Mean	S.D.
1	0	0	0	0	0	0	0			0	0
2	1.213	0.022	1.048	0.978	0.727	0.07	1.193	1.008	1.384	0.894	0.49
3	0	0	0	0	0					0	0
4	0.156	0.280	0.269							0.235	0.069
5	0.32	0.653	0.358	1.097	0.394					0.564	0.325

Table 2. Mean Group Tumor Volume (cm^3)

Group No.	1	2	3	4	5	6	7	8	9	Mean	S.D.
1	5.94	6.11	8.54	9.63	5.77	9.01	3.35			6.9	2.24
2	2.68	1.15	1.33	0.94	2.47	3.18	0.53	2.55	0.70	1.7	0.99
3	2.74	12.4	4.69	5.00	4.09					5.8	3.8
4	1.5	1.85	0.73							1.4	0.57
5	0.004	2.36	1.54	0.20	0.09					0.8	1.06

The volume of the solid tumor formed by the CEM cells is also measured to ensure that the reduction in p24 levels is not caused by failure of the tumor to transplant. Table 2 and Figure 2 demonstrate that the reduction in tumor volume is apparent in all groups treated with HIV but that the decrease in p24 serum levels is not correlated with the tumor volume (see virus control group No.2). Therefore, the reduction in p24 serum levels is not due to the small size of the solid tumor but to a real effectiveness of AZT or Rapamycin on the replication of HIV. The reduction in size of the tumor is rather explained by the infection of the virus in the CEM cells.

EXAMPLE 2.

***In vitro* incubation of rapamycin with CD4+ human cells uninfected and infected with defective HIV-1.**

TEST CELL CULTURES:

Different concentrations of rapamycin were tested on the following cell lines in culture:
-MT-4 ($CD4^+$ T lymphocytes);
-MT-2 (MT-4 cells infected with a defective HIV-1 HTLV III_B [Harada *et al.*, Science 229: 563-566, 1985, enclosed herewith by reference];
-U937 (monocyte) (ATCC CRL-1593); and
-UHC8 (U937 infected with a defective HIV-1 HTLV III_B, R3 strain [Boulerice *et al.*, J. Virol., 64, 1745-1755, 1990, enclosed herewith by reference].

Maintenance media.
All culture cells were maintained in RPMI-1640 medium with 10% heat-inactivated fetal calf serum and 5U/5µg/mL penicillin/streptomycin. The cells were propagated at 37°C in a humid atmosphere.

Preparation of compounds.
Concentrations of rapamycin were freshly prepared before incubation with cells. Serial dilutions were made from a concentrated solution of 1 mg/mL in ethanol and 100 µl were added to each well (final concentration of EtOH less than 0.0002%). 100 µl of cell suspension at 1×10^6 cell/mL was added to obtain 1×10^5 cells/well and drug concentrations of: 0, 0.01, 0.1, 1, 10, and 100 ng/mL.

EXPERIMENTAL DESIGN:
Cells were incubated in the presence or absence of drug for 48 hours before adding 1 µCi/well of ^{3}H-thymidine. After 6 hours further incubation, cells were harvested on

a glass fiber filter (Wallac) and washed 5 times with H_2O. Filters were then counted on a β-counter.

Results are presented in Table 3 as percent of inhibition of cellular growth of infected and uninfected host cells:

Table 3

Rapamycin (ng/ml)	MT-4 (% inhibition)	MT-2 (% inhibition)	U-937 (% inhibition)	UHC8 (% inhibition)
0	0	0	0	0
0.01	39	49	24	48
0.1	78	85	78	83
1	87	–	–	87
10	81	85	78	81
100	82	86	82	82

The toxicity of the compound at 100 ng/ml through 0.1 ng/ml was greater than about 80% cellular inhibition both for uninfected and infected cells. Although, at 0.01 ng/ml, the infected cells seemed slightly more sensitive to rapamycin than the uninfected cells, it seems that the anti-HIV effect of rapamycin may be due primarily to its toxicity on the replication of the host cells (lymphocytes and monocytes).

EXAMPLE 3.

IN VITRO EVALUATION OF RAPAMYCIN IN THE EXTRACELLULAR p24 ANTIGEN ASSAY AGAINST HIV TYPE 1 IN ACUTELY INFECTED CCRF-CEM AND U937 CELLS.

This assay is to determine the *in vitro* efficacy and cytotoxicity of rapamycin when the CEM and U-937 are acutely infected with HIV strain HTLV III_B, treated with the compound and the supernatants assayed for p24 antigen.

Preparation of compounds.

Rapamycin was suspended in DMSO to 30 mg/ml. Initial dilutions from this stock solution were also prepared in DMSO. Subsequent dilutions were prepared in medium to achieve final concentrations of 1000, 100, 10, 1, 0.1, 0.01, and 0.0001 ng/ml in 1% DMSO. The diluted compounds were soluble and prepared on the day of use. An AZT control was set up in parallel at the following concentrations: 1000, 100, 10, 1, 0.1, 0.01, and 0.001 ng/ml in 1% DMSO. Control cultures received 1% DMSO.

Infection and preparation of cells.

CCRF-CEM and U-937 cells were acutely infected with virus and a MOI of 0.1, and adsorbed for 1 hour at 37°C in 5% CO_2. Uninfected cells for controls were prepared with media in parallel with infected cells. After the 1 hour adsorption time, the cells were washed once with media by centrifugation to remove unadsorbed virus. The uninfected cells were also centrifuged. Cells were resuspended with the appropriate volume of medium to yield 5 x 10^4 cells/100 µl.

Each well received 100 µl of media-DMSO 2% or 2x compound and 100 µl of cells (infected or uninfected) to make a total volume of 200µl.

Incubation and determination of viral infectivity.

The microtiter plates were incubated at 37°C in humidified air with 5% CO_2. Since the cells do not display CPE or lyse after infection, preliminary immunofluorescence antibody (IFA) studies were conducted on virus control cells before terminal HIV-1 p24 gag protein measurements were performed by Enzyme Immunoassay (EIA) to insure proper infection. On day 6, approximately 25% of the cells were infected as observed by IFA. On day 8, approximately 40% of the cells were infected.

Determination of antiviral activity and cytotoxicity. On day 8, supernatants were collected for extracellular p24 measurements to determine efficacy. A microculture tetrazolium assay (MTA), using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-H-tetrazolium hydroxide (XTT), was also performed to determine cytotoxicity of the compound. The MTA detects the presence of viable cells. When compared to untreated cells, the percent of cellular proliferation of the treated cells can be quantitated. A decrease in percent cellular proliferation may be due to either cell stasis or cell death.

<u>CCRF-CEM CELLS TREATED WITH:</u>

a) Rapamycin:
Data of tests on CCRF-CEM cells acutely infected with HIV-1 incubated with 8 concentrations of rapamycin are shown in Figure 3. The percent cellular proliferation of the cells treated with 1000 ng/ml of the compound was 13.5%. As the concentration decreased cellular proliferation increased. The cytotoxic concentration at 50% (TC_{50}) is 0.6494 ng/ml. At concentrations of 0.1 ng/ml and below, cellular proliferation approached that of the untreated cells.

The efficacy of the compound at 1000 ng/ml through 1 ng/ml was greater that 95% viral inhibition. At 0.1 ng/ml the inhibitory effect of the compound declined (50.1% viral inhibition) to produce a sigmoidal dose-response curve with an effective concentration at 50% (EC_{50}) of 0.0965 ng/ml (Figure 3).

b) AZT (control):
Data of tests on CCRF-CEM cells acutely infected with HIV-1 incubated with 7 concentrations of AZT are shown in

Figure 4. The cellular proliferation of the cells treated with AZT throughout the concentration range approached that of the untreated cells.

The efficacy of AZT at 1000 ng/ml through 100 ng/ml was greater than 99% viral inhibition. At 10 ng/ml the inhibitory effect declined (85.9% viral inhibition) to produce a sigmoidal dose-response curve with an effective concentration at 50% of 1.0 ng/ml.

The antiviral effect of Rapamycin is likely due to its antiproliferative effect on CCRF-CEM cells. The graph in Figure 3 shows that as the antiproliferative effect decreases, the antiviral effect also declines.

<u>U937 CELLS TREATED WITH:</u>

a) Rapamycin:

Data of tests on U937 cells acutely infected with HIV-1 incubated with 8 concentrations of rapamycin are shown in Figure 5. The percent cellular proliferation of the cells treated with 1000 ng/ml of the compound was 23.3%. As the concentration decreased cellular proliferation increased. The cytotoxic concentration at 50% (TC_{50}) is 2.105 ng/ml. At concentrations of 0.1 ng/ml and below, cellular proliferation approached that of the untreated cells.

The efficacy of the compound at 1000 ng/ml through 1 ng/ml was greater that 95% viral inhibition. At 0.1 ng/ml the inhibitory effect of the compound declined (73.8% viral inhibition) to produce a sigmoidal dose-response curve with an effective concentration at 50% (EC_{50}) of 0.0172 ng/ml (Figure 5).

<u>b) AZT (control):</u>

Data of tests on U937 cells acutely infected with HIV-1 incubated with 7 concentrations of AZT are shown in Figure 6. The cellular proliferation of the cells treated with

AZT throughout the concentration range approached that of the untreated cells.

The efficacy of AZT at 1000 ng/ml through 100 ng/ml was greater than 85% viral inhibition. At 10 ng/ml the inhibitory effect declined (60.7% viral inhibition) to produce a sigmoidal dose-response curve with an effective concentration at 50% of 2.4 ng/ml.

The antiviral effect of Rapamycin is likely due to its antiproliferative effect on U937 cells. The graph in Figure 4 shows that as the antiproliferative effect decreases, the antiviral effect also declines.

Although the results presented herein are based on treatment with rapamycin, it can be reasonably predicted by any person skilled in the art that analogs of rapamycin possessing similar immunosuppressive activity would also possess similar or comparable anti-HIV activity. It is therefore a further aspect of this invention to use analogs of rapamycin in the manufacture of a medicament for the treatment of $CD4^+$ cells infection, particularly by HIV.

WHAT IS CLAIMED IS:

1. Use of rapamycin or an analog thereof in the manufacture of a medicament for treating, arresting the development or retarding the progression of an HIV infection in an mount sufficient to achieve a reduction in the level of serum p24 antigen.

2. The use according to claim 1, wherein said rapamycin analog is selected from mono- or diacyl rapamycin; hydrogenated derivatives of rapamycin; 42-oxorapamycin; 27-oxime rapamycin; rapamycin pro-drugs; 42-oxime rapamycin; silyl esters of rapamycin; rapamycin dimers; rapamycin hydrazone; bicyclic rapamycin; carbamates of rapamycin; amide esters of rapamycin; 15-hydroxy- and 15,27-hydroxyrapamycin; carboxylic acid esters of rapamycin; fluorinated esters of rapamycin; 29-desmethyl rapamycin; or 7,29-bisdesmethyl rapamycin.

3. The use according to claim 1, wherein said medicament further comprises another anti-HIV agent.

4. The use according to claim 3, wherein said other anti-HIV agent is a reverse transcriptase inhibitor.

5. The use according to claim 4, wherein said reverse transcriptase inhibitor is selected from AZT, d4T, ddC, ddI, 3TC or analogs of 3TC.

6. The use according to claim 5, wherein said reverse transcriptase inhibitor is present in an amount effective to inhibit HIV replication.

7. The use claimed in any one of claims 1 to 6, wherein the medicament is adapted for oral administration.

8. The use claimed in any one of claims 1 to 6, wherein the medicament is adapted for parenteral administration.

9. The use claimed in any one of claims 1 to 6, wherein the medicament is in unit dosage form.

10. The use claimed in any one of claims 1 to 6, wherein said rapamycin or analog thereof is present in said medicament to administer a dose ranging from 0.25 to 500 mg/kg of body weight per day.

11. The use according to claim 10, wherein said rapamycin or analog thereof is present in said medicament to administer a dose ranging from 5 to 500 mg/kg of body weight per day.

12. The use according to claim 11, wherein said rapamycin or analog thereof is present in said medicament to administer a dose ranging from 10 to 250 mg/kg of body weight per day.

13. A method for treating, arresting the development or retarding the progression of AIDS in a mammal in need thereof which comprises administering to said mammal an amount of rapamycin or analog thereof sufficient to achieve a reduction in the level of serum p24 antigen.

14. The method according to claim 13, wherein said rapamycin analog is selected from mono- or diacyl rapamycin; hydrogenated derivatives of rapamycin; 42-oxorapamycin; 27-oxime rapamycin; rapamycin pro-drugs; 42-oxime rapamycin; silyl esters of rapamycin; rapamycin dimers; rapamycin hydrazone; bicyclic rapamycin; carbamates of rapamycin; amide esters of rapamycin; 15-hydroxy- and 15,27-hydroxyrapamycin; carboxylic acid esters of rapamycin; fluorinated esters of rapamycin; 29-desmethyl rapamycin; or 7,29-bisdesmethyl rapamycin.

15. A method according to claim 13, wherein said rapamycin or analog thereof is administered orally or parenterally.

16. The method according to claim 13, further comprising administering said rapamycin or analog thereof in combination with another anti-HIV agent.

17. The method according to claim 16, wherein said other anti-HIV agent is a reverse transcriptase inhibitor.

18. The method according to claim 17, wherein said reverse transcriptase inhibitor is selected from AZT, d4T, ddC, ddI, 3TC or analogs of 3TC.

19. The method according to claim 18, wherein said reverse transcriptase inhibitor is administered in an amount effective to inhibit HIV replication.

20. The method according to anyone of claims 13 to 19 wherein said rapamycin or analog thereof is administered in a dose ranging from 0.25 to 500 mg/kg of body weight per day.

21. The method according to claim 20, wherein said rapamycin or analog thereof is administered in a dose ranging from 5 to 500 mg/kg of body weight per day.

22. The method according to claim 21, wherein said rapamycin or analog thereof is administered in a dose ranging from 10 to 250 mg/kg of body weight per day.

23. Use of rapamycin or analog thereof for treating, arresting the development or retarding the progression of AIDS in a mammal.

24. Use of rapamycin according to claim 23, wherein said rapamycin analog is selected from mono- or diacyl rapamycin; hydrogenated derivatives of rapamycin; 42-oxorapamycin; 27-oxime rapamycin; rapamycin pro-drugs; 42-oxime rapamycin; silyl esters of rapamycin; rapamycin dimers; rapamycin hydrazone; bicyclic rapamycin; carbamates of rapamycin; amide esters of rapamycin; 15-hydroxy- and 15,27-hydroxyrapamycin; carboxylic acid esters of rapamycin; fluorinated esters of rapamycin; 29-desmethyl rapamycin; or 7,29-bisdesmethyl rapamycin.

25. A method for treating, arresting the development or retarding the progression of AIDS in a mammal in need thereof which comprises administering to said mammal an anti-HIV effective amount of rapamycin or an analog thereof.

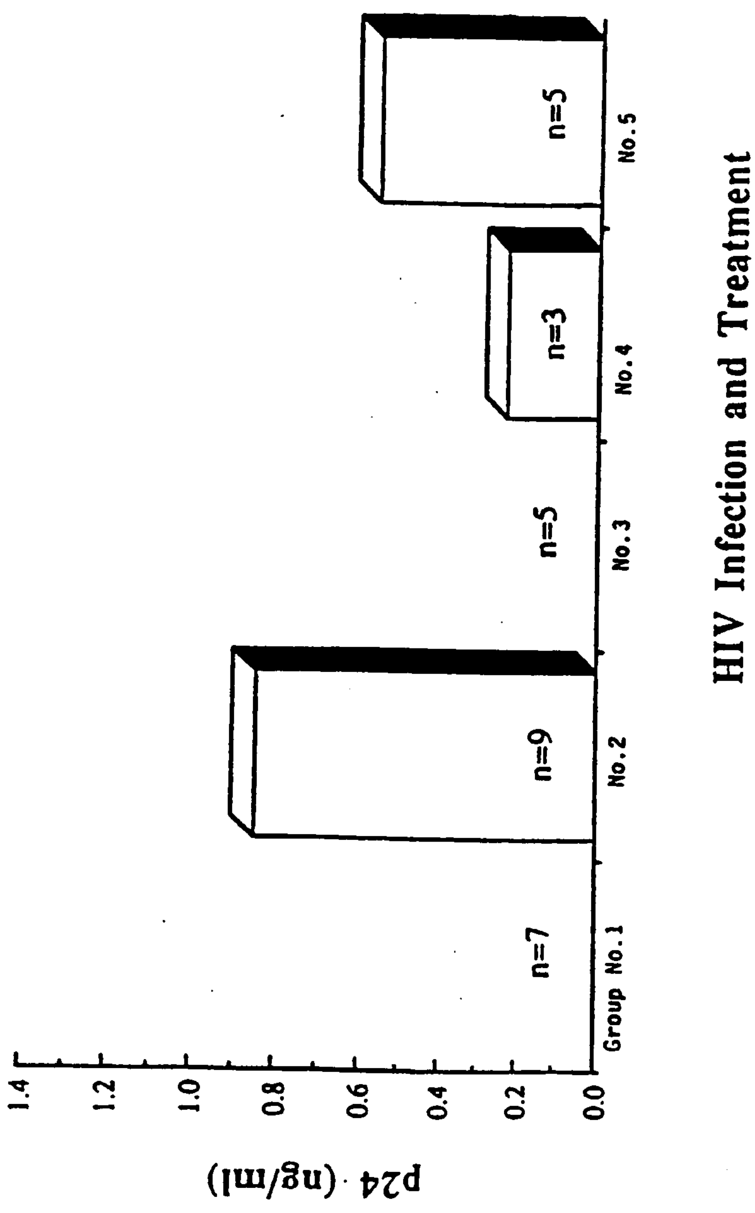
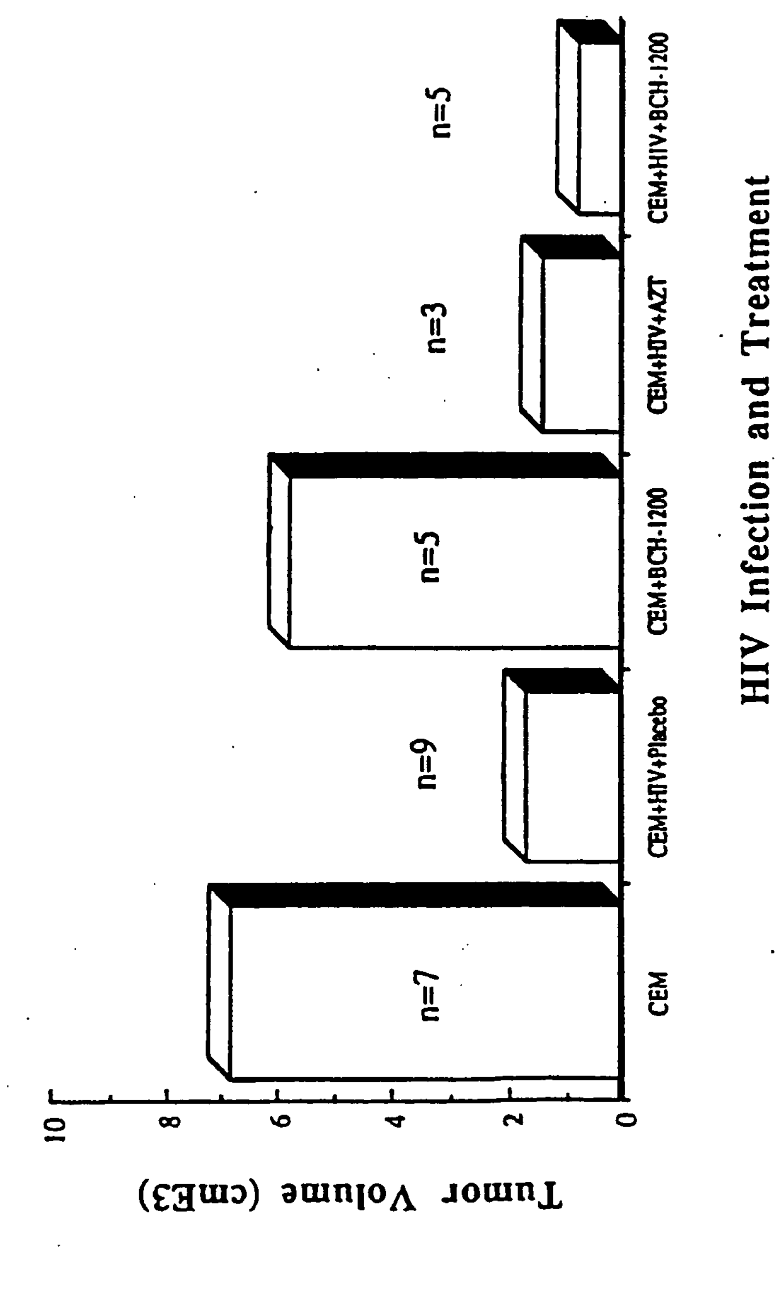

HIV Infection and Treatment
p24 (ng/ml)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Group No.1
No.2
No.3
No.4
No.5
n=7
n=9
n=5
n=3
n=5

FIGURE 1


Tumor Volume (cmE3)
0
2
4
6
8
10
n=7
n=9
n=5
n=3
n=5
CEM
CEM+HIV+Placebo
CEM+BCH-1200
CEM+HIV+AZT
CEM+HIV+BCH-1200
HIV Infection and Treatment

2 / 6

FIGURE 2

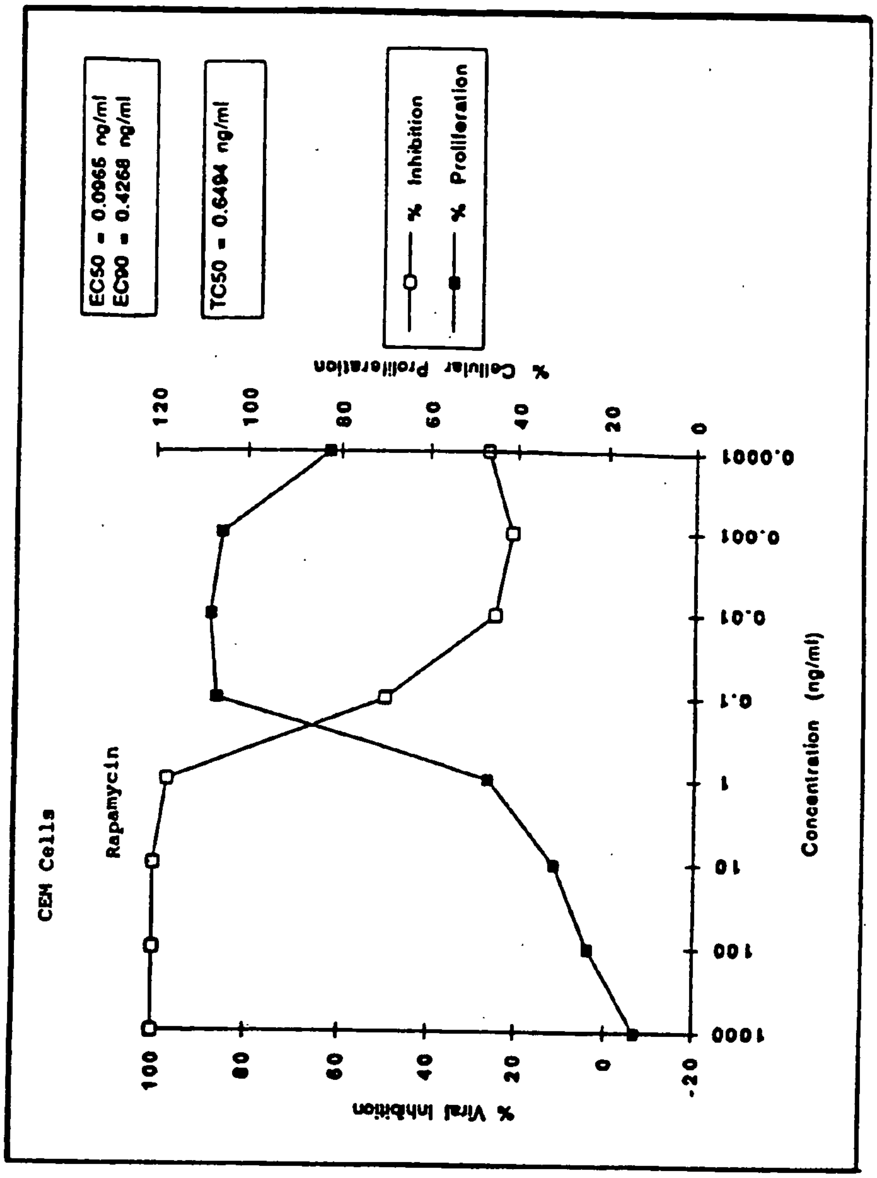

EC50 = 0.0966 ng/ml
EC90 = 0.4268 ng/ml
TC50 = 0.6494 ng/ml
% Inhibition
% Proliferation
CEM Cells
Rapamycin
% Cellular Proliferation
% Viral Inhibition
Concentration (ng/ml)
0.0001
0.001
0.01
0.1
1
10
100
1000

FIGURE 3

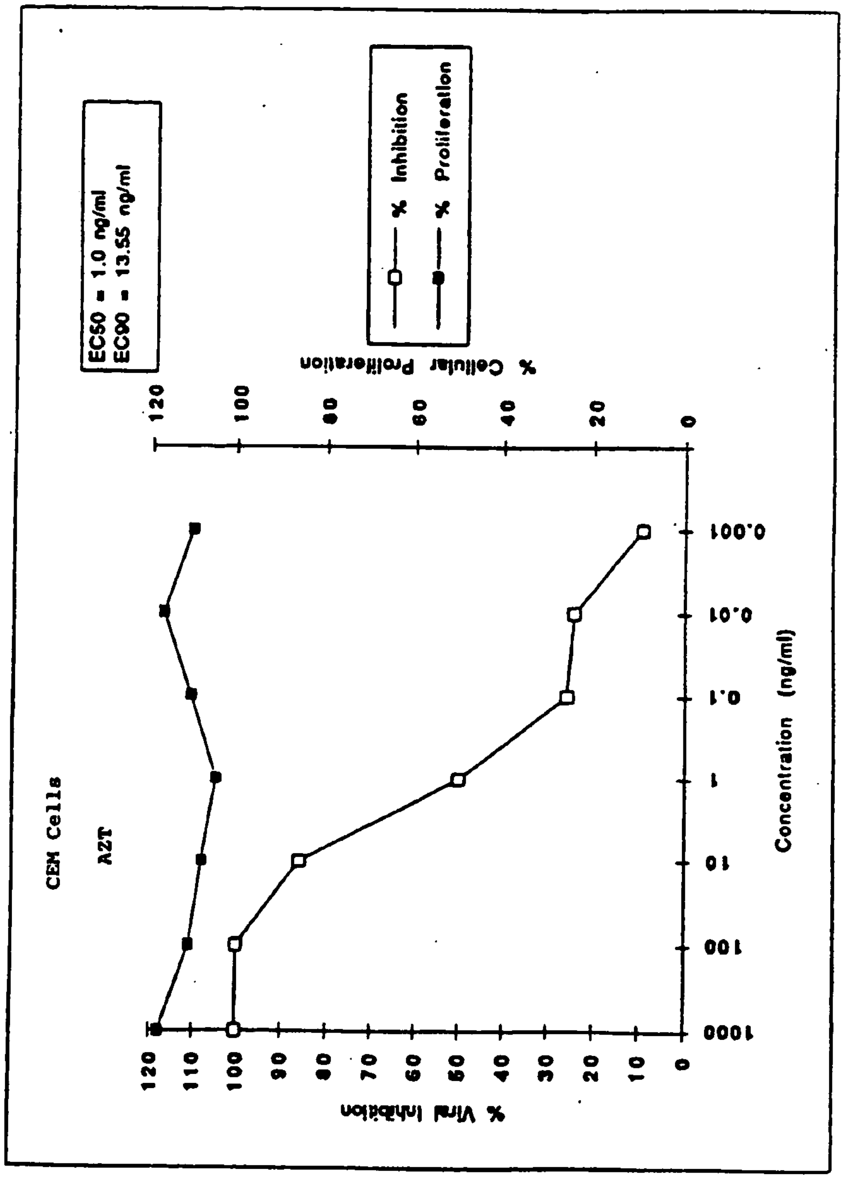

CEM Cells
AZT
EC50 = 1.0 ng/ml
EC90 = 13.55 ng/ml
% Inhibition
% Proliferation
% Cellular Proliferation
% Viral Inhibition
Concentration (ng/ml)
1000
100
10
1
0.1
0.01
0.001
120
110
100
90
80
70
60
50
40
30
20
10
0

FIGURE 4

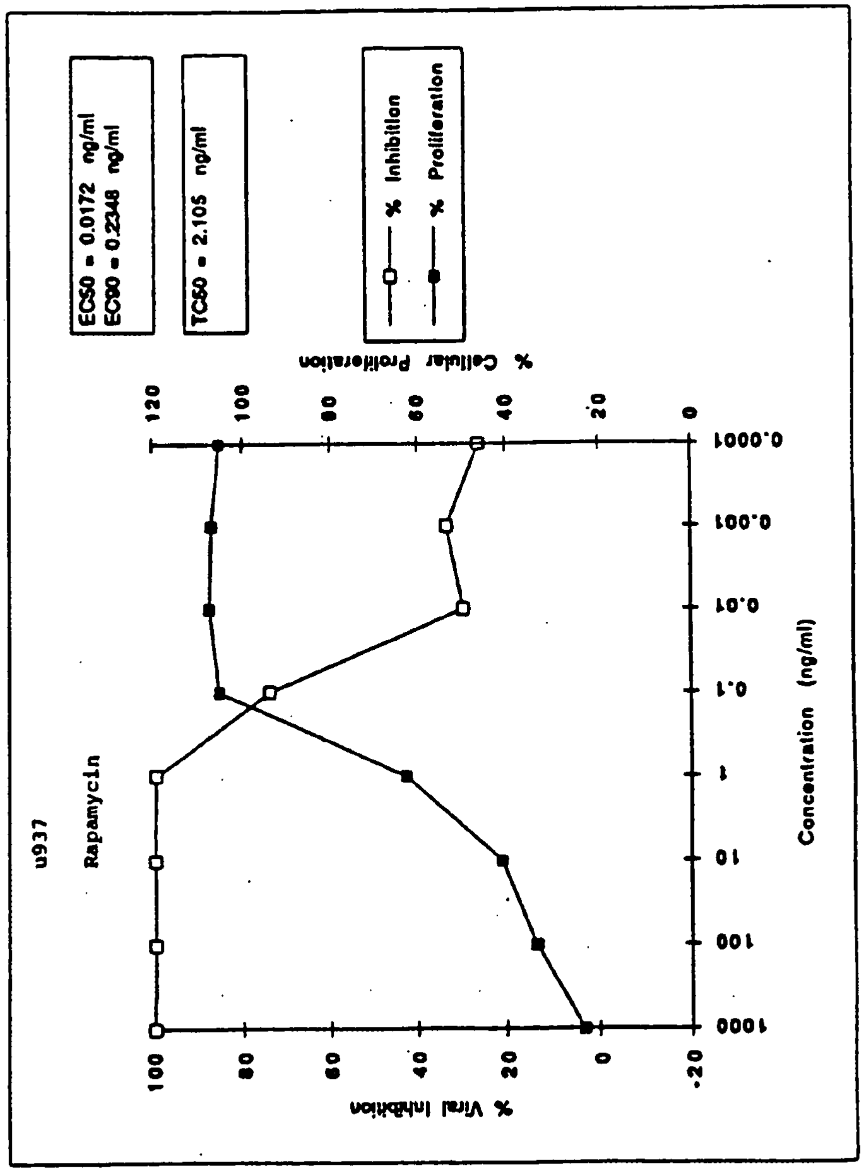

u937
Rapamycin
EC50 = 0.0172 ng/ml
EC90 = 0.2348 ng/ml
TC50 = 2.105 ng/ml
% Inhibition
% Proliferation
% Viral Inhibition
% Cellular Proliferation
Concentration (ng/ml)
100
80
60
40
20
0
-20
120
0.0001
0.001
0.01
0.1
1
10
100
1000

FIGURE 5

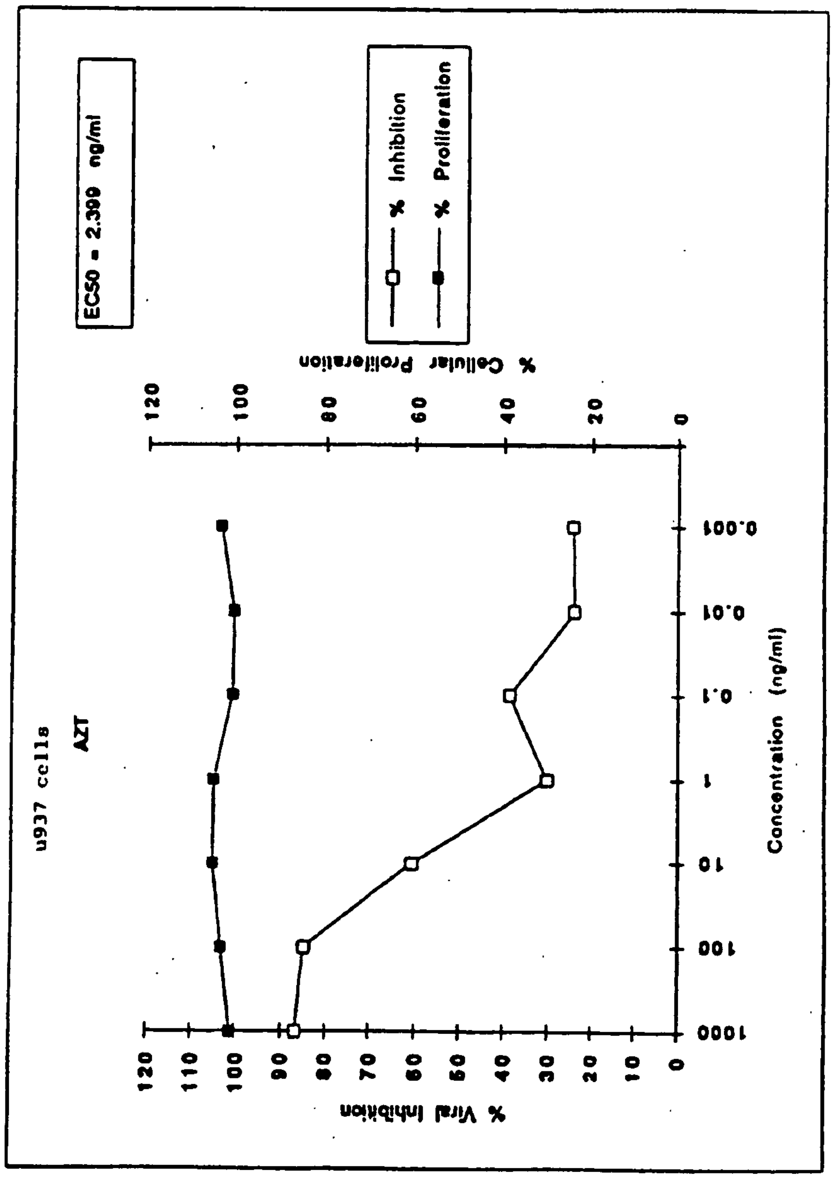


FIGURE 6

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/[illegible]3/00384

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/71

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 093 338 (BYRNE ET AL.) 3 March 1992 cited in the application see the whole document	1,7-14, 20-25
X	US,A,5 091 389 (ONDEYKA ET AL) 25 February 1992 cited in the application see the whole document	1,7-14, 20-25
A	J. NEUROIMMUNOL. vol. 0, no. SP 1 , 1991 page 201 P. HÖLLSBERG ET AL. 'HTLV-1 induced spontaneous T-cell clonal proliferation is rapamycin sensitive'	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1993	28.12.93.

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Klaver, T

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/CA 93/00384

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5093338	03-03-92	EP-A-	0510904	28-10-92
		JP-A-	5186477	27-07-93
US-A-5091389	25-02-92	EP-A-	0510903	28-10-92
		JP-A-	5153991	22-06-93